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14. ABSTRACT
Studies in year one have shown that the rat dorsal burn wound model is appropriate for testing the analgesic and healing rate efficacy of test drugs. This model was used to test Indomethacin-PC (Indo-PC), Indomethacin and appropriate controls when administered either topically or parenterally. The experimental drug Indo-PC showed significant analgesic efficacy over Indo alone at an early time point when the drugs were administered subcutaneously, and Indo-PC also tended to provide better analgesia than Indo alone after topical administration. No differences in healing rate were evident from any treatment. These studies support a rationale for continued development of Indo-PC for treatment of acute pain caused by 2nd degree burn wounds.

15. SUBJECT TERMS
Burn, phospholipid, NSAID, topical, pain, inflammation

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The major problem under study is the development of a topical or parenteral treatment for pain due to 2nd degree burn injury. The experimental drug treatment consists of the use of nonsteroidal anti-inflammatory drugs (NSAIDs) that are complexed with the phospholipid phosphatidylcholine (PC) to produce a new class of drugs, NSAID-PCs, which have been shown in other experimental systems to reduce inflammation and pain and promote healing. This grant will utilize rodent models of burn injury to test the efficacy of indomethacin-PC (Indo-PC) and ibuprofen-PC (Ibu-PC) versus unmodified indomethacin (Indo) and ibuprofen (Ibu) when administered either topically or subcutaneously. Measurements of pain and wound healing will be made. After these drugs are fully tested in preclinical systems and shown to be beneficial, they can be further developed for clinical use.

KEYWORDS

Burn
Phospholipid
NSAID
Topical
Pain
Inflammation

ACCOMPLISHMENTS

Major goals of the project:

1) Compare the efficacy of topical Indomethacin-PC and Ibuprofen-PC in rodent models of 2nd degree burn injury, and determine any additional benefit of combined topical and parenteral administrations.
2) Evaluate the GI side effects of PC-NSAID treatment in the burn model and the effect of test drugs on clotting time.
3) Determine the mechanism of action of PC-NSAIDs in the treatment of burn pain/healing.

Milestones and target dates:

1) Complete testing for topical and iv Indo, Indo-PC, Ibu and Ibu-PC in hind limb burn injury model – target was month 6 – 0% complete
2) Complete testing for topical and iv Indo, Indo-PC, Ibu and Ibu-PC in dorsal skin burn injury model – target was month 12 – 50% complete
3) Complete testing for topical vs iv NSAID-PCs in hind limb burn injury model – target was month 15 – 0% complete
4) Complete testing for topical vs iv NSAID-PCs in dorsal skin burn injury model – target was month 18 – 0% complete

5) Determination of NSAID-induced GI side effects in hind limb burn injury model – target was month 15 – 0% complete
6) Determination of NSAID-induced GI side effects in dorsal skin burn injury model – target was month 18 – 50% complete
7) Determination of effects of PC-NSAIDs on thrombus formation and clotting time – target was month 18 – 0% complete
8) Assessment of COX inhibition and effects on inflammation as a mechanism for PC-NSAID efficacy – target was month 24 – 0% complete
9) Assessment of skin hydrophobicity and histology as a mechanism for PC-NSAID efficacy – target was month 24 – 25% complete

Accomplishments

1) Major activities: During this first grant year, it was necessary to hire and train new personnel due to the retirement of former staff. It was also necessary to obtain ACURO approval for animal studies. With those issues resolved, studies on the dorsal burn model were initiated and many parts were completed. Quarterly reports and quad charts were prepared and submitted as required.
2) Specific objectives: To test topically and parenterally administered Indo and Indo-PC vs appropriate controls in the dorsal burn injury model for ability to relieve pain and promote wound healing.
3) Significant results: Because of the labor-intensive nature of these studies, only a few animals at a time could be tested. However, the basic study was repeated until an N of 9-11 for most groups was obtained. The results shown below are a compilation of all studies. The protocol consisted of performing baseline behavioral testing of rat hind paw withdrawal to von Frey filament stimulation (explained below). Then the animals were administered the dorsal burn wound under anesthesia and were maintained for two days with treatments of buprenorphine for acute pain. Experimental drugs treatments were begun two hours after initiation of the burn wound, and continued daily until the termination of the study. At days 3, 5, 10 and 15 post-burn, the animals were again tested for sensitivity to a stimulus (von Frey hair of varying stiffness) on a hind paw. This test was to determine whether any treatment could reverse burn-induced hyperalgesia (hypersensitivity to pain). Also at days 3, 5, 10 and 15 post-burn, the burn wound size was measured by caliper to determine the rate of healing of the wound. In addition at these times during the 15 day treatment period, samples of fecal pellets were collected for determination of hemoglobin as a measure of gastrointestinal (GI) bleeding into the intestinal lumen which is a potential side effect of NSAIDs. Finally, at euthanasia, the stomach, intestines and colon were examined macroscopically for signs of lesions or adhesions which are GI manifestations of NSAID-induced injury, and samples of the burn wound were processed for histological examination and evaluated by Dr. Roger Bick, Director of the Multi-user Fluorescence Imaging and Microscopy Core at the PI's institution. The results are presented below in several figures. Figure 1A and B represent the same von Frey testing data, but with emphases on different

significant effects. Figure 1 displays the withdrawal threshold to von Frey filaments as a percent of baseline for all the treatment groups: naïve (control, non-burn), PBS (burn injury plus subcutaneous vehicle (PBS)), Indo/PBS (burn plus subcutaneous Indo in PBS), Indo-PC/PBS (burn plus subcutaneous Indo-PC in PBS), Oil (burn plus topical oil), Indo/Oil (burn plus topical Indo in oil), and Indo-PC/Oil (burn plus topical Indo-PC in oil). Figure 1A shows that the PBS group (burn control) exhibited significant hyperalgesia on days 3 and 10 post-burn, seen as a reduction in the withdrawal threshold. This finding in controls is important to verify so that all the treatment groups can be compared to it. Thus, any treatment that reverses the hyperalgesia seen in PBS controls, is considered as providing pain relief. In Figure 1B, the only significant effect was seen with Indo-PC in PBS, where the withdrawal threshold was higher at day 3. *This result indicates that the subcutaneous Indo-PC provided significant pain relief at the early time post-burn.* It should be noted that most of the other treatment groups also were reversed to baseline values, but did not reach statistical significance like the Indo-PC group, likely due to the high variability that is inherent in behavioral testing with von Frey filaments. It is also of note that the Indo-PC treatments, both topical and subcutaneous, tended to be higher than their Indo alone counterpart, suggesting an apparent analgesic benefit to either Indo-PC treatment.

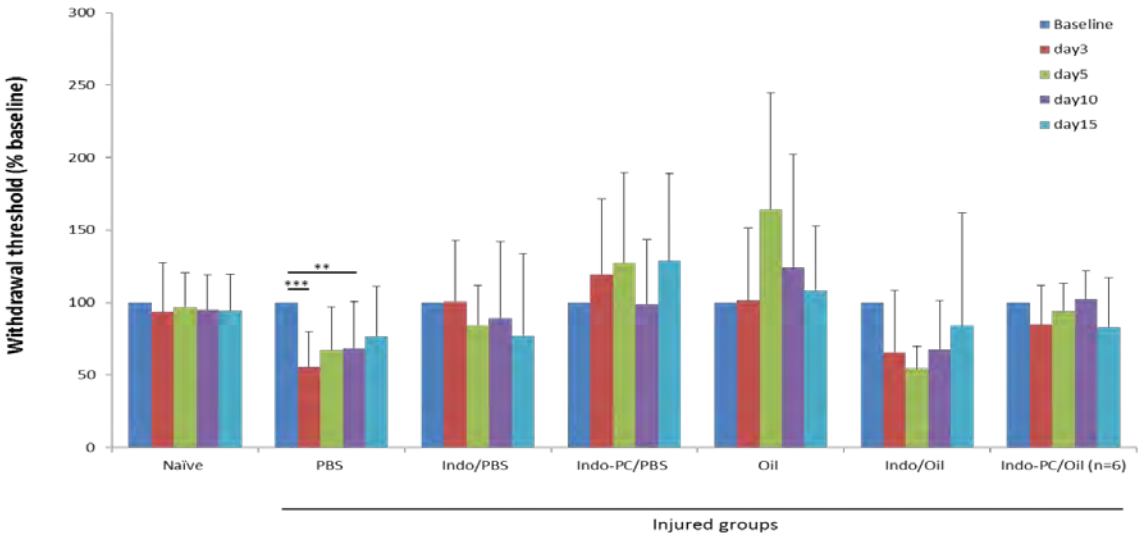

Figure 1A. Burn injury-induced hyperalgesia was present in PBS controls at days 3 and 10 post-burn. (**p<0.01; ***p<0.001)

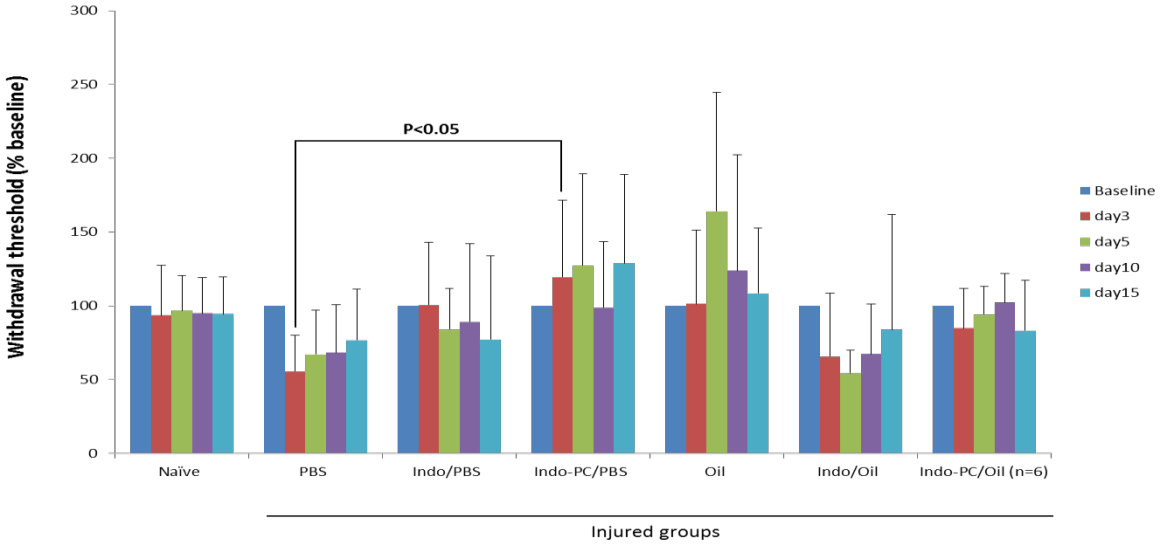

Figure 1B. Burn injury-induced hyperalgesia was significantly reduced by parenteral Indo-PC at day 3 post-burn.

The rate of burn wound healing for the same treatment groups as above is shown in Figure 2. It is seen that all groups exhibited similar patterns of reducing wound size over time, with no treatment showing better efficacy than the normal rapid rate of healing observed in PBS controls. These results suggest that none of the treatments showed a clear benefit with regard to the healing of 2nd degree burn injury. It is therefore conceivable that more severe burn injury needs to be studied to fully assess the potential benefit of Indomethacin-PC to accelerate wound healing.

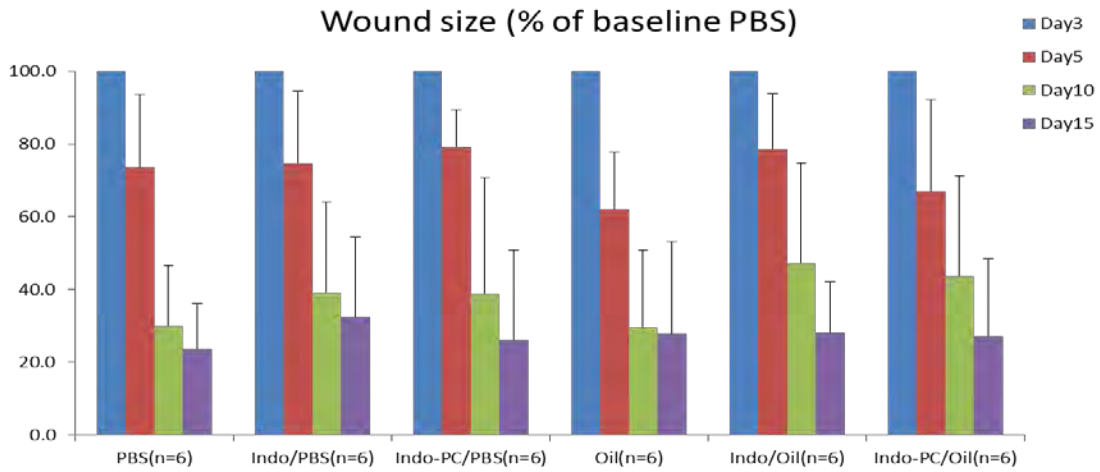

Figure 2. Burn wound healing rate was similar among treatment groups.

To further evaluate the wound healing, specimens were examined at a microscopic level. Examples of tissue intactness and surface injury are shown in Figure 3 where

naïve and burn samples revealed typical injury. It was found that all treatment groups exhibited areas of normal tissue as well as injured tissue, and that the histological evaluation gave a similar result as the measurement of wound size – no treatment showed a clear histological benefit in promoting skin healing, which is consistent with the caliper measurements described above.

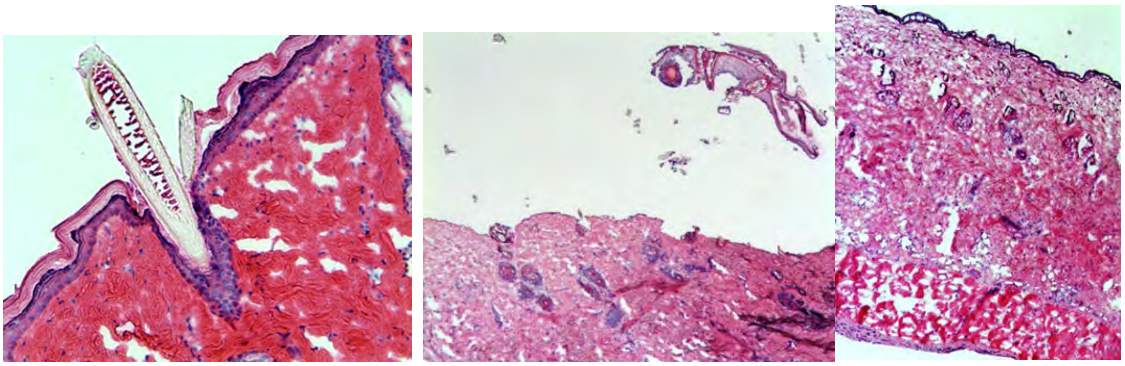

Figure 3. Histology examples of naïve (left) and burned dorsal skin (center and right) with loss of surface epidermis and collagen discoloration and rearrangement (center) and damage to the hair follicle, shaft and roots (right).

The potential for NSAID-induced GI bleeding was evaluated and is shown in two parts of Figure 4. The left figure shows bleeding into fecal pellets collected just prior to euthanasia at day 15, where no differences among groups are evident. The right figure shows the blood hematocrit at that time, where again, no differences among groups are seen. These results show that the doses of NSAID used, which reduced burn-induced hyperalgesia did not produce any clinically significant GI bleeding. These results are consistent with the finding of no lesions, adhesions or perforations upon examination of stomach and intestines at euthanasia in the animals administered the test NSAIDs either parenterally or topically.

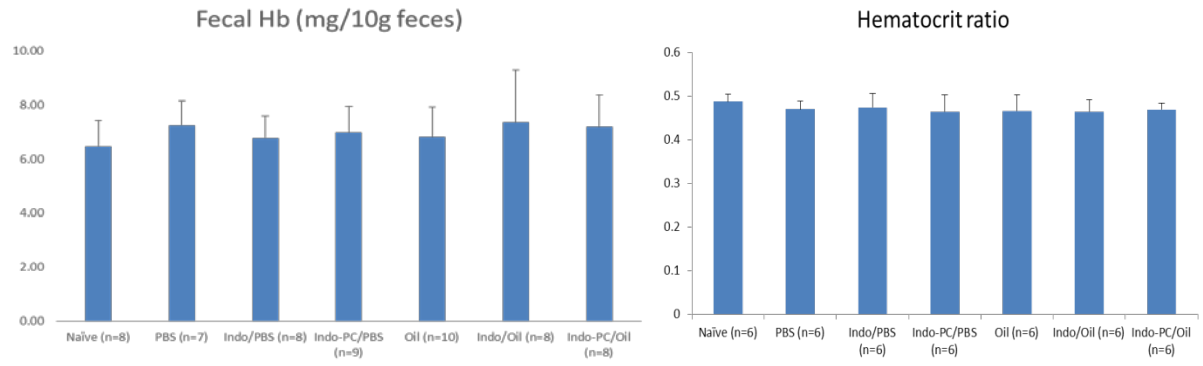

Figure 4. Potential NSAID-induced GI bleeding is not evident from either blood in fecal pellets (left) or hematocrit values (right).

In summary, studies in year one have shown that the rat dorsal burn wound model is appropriate for testing the analgesic and healing rate efficacy of test drugs. The experimental drug Indo-PC showed significant analgesic efficacy over Indo alone at an early time point when the drugs were administered subcutaneously, and Indo-PC also tended to be better than Indo alone after topical administration. No differences in healing rate were evident from any treatment.

Opportunities for training and professional development

Nothing to report.

Results dissemination

Nothing to report.

Goals for next reporting period

Studies will continue with the rodent burn model to test the efficacy of Indo, Indo-PC, Ibu, and Ibu-PC at relieving pain and promoting healing. Studies with Indo and Indo-PC are close to completion and studies with Ibu and Ibu-PC will commence soon.

IMPACT

Impact on principal discipline (pharmacological treatment of 2nd degree burns)

Results to date using animal models support further research into the use of parenteral and topical Indo-PC for treating 2nd degree burns. This product may provide analgesia while burn wounds are healing.

Impact on other disciplines

The topical and parenteral products under study may find applications in other areas of (non-burn) wound pain suppression or healing.

Impact on technology transfer

The topical and parenteral products under study are covered in patents held jointly by Dr. Lichtenberger and The University of Texas Health Science Center at

Houston, and licensed to PLx Pharma LLC of Houston TX. PLx is in a position to develop the products for the US and global markets.

Impact on society

Nothing to report.

CHANGES/PROBLEMS

Nothing to report.

PRODUCTS

Nothing to report.

PARTICIPANTS & OTHER COLLABORATION ORGANIZATIONS

Project individuals

Name	Lenard Lichtenberger	Dexing Fang	Kaori Ono	Tri Phan
Project role	Principal Investigator	Senior Research Scientist	Research Associate	Senior Research Assistant
Researcher identifier				
Person-month worked	1.1	1.29	5.4	3.7
Contribution to project	Planned and directed all studies; wrote reports	Supervised staff; analyzed data	Performed animal studies including wound induction, drug dosing, behavioral testing and histological preparation	Assisted with burn wound induction and analyzed hematocrit and hemoglobin in fecal pellets
Funding support	This grant; NIH grants; state of Texas funds	This grant; NIH grants; state of Texas funds	This grant; NIH grants; non-profit grants	This grant; NIH grants; state of Texas funds

Change in active other support of the PI

Lenard M. Lichtenberger

Closed Support

1R03CA171613 (PI: Lichtenberger) 09/03/2012-08/31/2014
NIH/NCI 0.6 calendar mo. $50,000/yr
PC-NSAIDs for Chemoprevention of Colorectal Cancer
The major goal of this grant is to test Ibuprofen-PC and Indomethacin-PC for chemopreventive activity in an animal model of colon cancer.
Role: Principal Investigator
Budgetary & Scientific Overlap: None

2 R44HD061132 (PI: Marathi) 04/01/2012-03/30/2015
NIH/NICHD 1.2 calendar mo. $122,000/yr
GI-Safer Formulation of Indomethacin for Use in Preterm Neonates
The major goal of this SBIR Phase II grant is to develop an intravenous formulation of indomethacin-PC for use in closing patent ductus arteriosus in premature infants.
Role: Co-Investigator
Budgetary and Scientific Overlap: None

1R41CA171408 (PI: Lichtenberger) 05/03/2013-04/30/2014
NIH/NCI 1.2 calendar mo. $85,000/yr
Aspirin-PC for Chemoprevention of Colorectal Cancer
The major goal of this Phase I STTR grant is to test aspirin-PC for chemopreventive activity in an animal model of colon cancer.
Role: Principal Investigator
Budgetary and Scientific Overlap: None

New Active Support

1R21CA182798-01A1 (PI, Lichtenberger) 04/01/2014-03/31/2016
NIH/NCI 1.2 calendar months $155,000/yr
Effects of anti-platelet drugs on colon cancer in the elderly
The major goal of this R21 grant is to test Aspirin-PC and other anti-platelet drugs on platelets collected from animals and human subjects of differing age and assessing the drugs' chemopreventive activity in an animal model of colon cancer.
Role: Principal Investigator
Budgetary and Scientific Overlap: None

MDACC Moon Shot (PI, Lichtenberger) 10/22/2014-10/21/2015

MD Anderson Cancer Center at Houston 0.24 calendar months $25,000/yr
Evaluation of the chemopreventive activity of Aspirin-PC, using in vitro and in vivo models of colorectal cancer (CRC)
The major goal of this small grant is to evaluate Aspirin-PC for activity against colorectal cancer.
Role: Principal Investigator of UT Core
Budgetary and Scientific Overlap: None

MDACC Ovarian SPORE (PI, Lichtenberger) 09/01/2014-08/31/2015
MD Anderson Cancer Center at Houston 0.24 calendar months $23,100/yr
Use of Aspirin-PC alone and in combination with chemotherapeutic agents to treat ovarian cancer
The major goal of this small grant is to evaluate Aspirin-PC in combination with other chemotherapeutic agents for activity against ovarian cancer.
Role: Principal Investigator of UT Core
Budgetary and Scientific Overlap: None

1P30DK56338 (Center PI: Estes) 03/01/13-02/28/18
NIH/NIDDK 10% effort $113,000/yr
Center for Gastrointestinal Development, Infection & Injury
Silvio O. Conte Digestive Diseases Research Center Core
The major goal of the Integrative Biology Core is to educate and train members of the Digestive Diseases Center in the development of animal models of digestive diseases.
Role: Director of Integrative Biology Core; Associate Director of the Center.
Budgetary and Scientific Overlap: None

<u>Other organizations as partners</u>

Nothing to report